Understanding Carabelli Expression by Sex and Population Through the Patterning Cascade Model of Tooth Morphogenesis

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Abstract

Carabelli's cusp is an accessory cusp seen in adult humans most frequently on the upper first molar. It has been widely studied in the anthropological literature and has come to be associated with greater frequency in Caucasian and African populations than in Asian and Native American populations. The cusp's sexual dimorphism has also often been a subject of dispute.

A 2010 study by Hunter and colleagues examined the developmental processes that lead to the morphological conditions under which Carabelli's cusp is expressed. Following a model of tooth morphogenesis proposed in a 2002 paper by Salazar-Ciudad and Jernvall, Hunter and colleagues examined the relationship between cusp spacing, crown area, and Carabelli expression. The study found that as intercusp spacing decreases, relative to tooth size, Carabelli expression increases. These findings support the predictions of the developmental model proposed by Salazar-Ciudad and Jernvall.

This study expands on the findings of Hunter et al. to examine how population and sex, as variables, along with intercusp spacing and crown area, correlate with Carabelli development. The results confirm the importance of intercusp spacing, relative to tooth area, as a predictor of Carabelli expression. The results also show that population does not significantly correlate with Carabelli development. Sex, however, does have a significant relationship to Carabelli expression, although likely to a lesser extent than intercusp spacing.

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INTRODUCTION

Carabelli's trait is an accessory cusp observed in varying degrees in modern humans, our ancestors, and primates. Georg von Carabelli (Fig. 1), an Austrian dentist after whom the cusp is named, described the cusp in detail in 1842 (Hillson, 2002). The cusp, or perhaps more accurately cingular and cuspule developments analogous to the cusp, are seen in primates including *Sivapithecus, Gigantopithecus, Gorilla, Pan,* and *Pongo* (Korenhof, 1960). It is present in hominins back through the Australopithecines (Turner and Hawkey, 1998). In modern adult humans, the cusp most frequently occurs on the permanent upper first molar, specifically as an extension of the mesiolingual cusp, or protocone (Dahlberg, 1945; Hillson, 2002). It ranges in development from a small groove or pit to a fully formed, free cusp whose size may come close to that of the main cusps (Fig. 2A and Fig. 2B).

Carabelli genetics

Researchers originally believed that Carabelli's cusp follows simple Mendelian inheritance rules. Some determined that the cusp is either simple autosomal dominant (Tsuji, 1958) or simple autosomal codominant (Kraus, 1951; Turner, 1967). In a study of dental casts from 123 white families, Goose and Lee (1971) used statistical analyses, assuming Hardy-Weinberg equilibrium, to show that dominant/recessive and codominant models are poor fits for Carabelli's cusp. They determined that the cusp is likely under multifactorial genetic control (Goose and Lee, 1971). Harris (1977) also concluded that Carabelli's trait cannot be explained by simple modes of inheritance. He suggested that tooth crown traits are quasi-continuous, a type of trait expression that will be discussed later, and that they have polygenic modes of inheritance. Since Carabelli's trait is quasi-continuous, the same factors that control inheritance of continuous traits, including genetic variation, shared environmental factors, and individual environmental factors, affect Carabelli (Hillson, 2002).

Several studies have attempted to quantify heritability for Carabelli's cusp. Townsend and Martin (1992) estimated heritability to be 0.94 for the left and 0.86 for the right permanent maxillary first molars. According to Scott and Turner (1997), these estimates could mean that both additive genes at various loci and a major dominant gene mainly affect Carabelli expression, while environmental influence is secondary to these genetic factors. This conclusion fits well with the patterning cascade model of the cusp, discussed later.

Nonmetric dental anthropology

Nonmetric dental traits are structures that are difficult to measure quantitatively, and therefore they are often recorded for either presence/absence or they are given a grade based on an ordinal scoring system. They include features like cusps, ridges, and fissures. Various systems have been used in the past, but currently the Arizona State University dental anthropology system (ASUDAS) is the most widely used and considered to be the standard (Hillson, 2002). Turner and colleagues developed the Arizona State University dental anthropology system in 1991.

Nonmetric studies of Carabelli's cusp are common throughout anthropological literature. Current nonmetric studies commonly use an eight-point scale developed by the ASUDAS. The scale (Fig. 3) is based on the classification systems of Dahlberg (1963), Hanihara (1963), and Scott (1980) (Hillson, 2002). It ranges in point value from zero to seven, with zero being complete absence of the trait, one being a slight groove, two being a pit, three being a shallow Y-shaped groove, four being a deep Y-shaped groove, five being a small cusp with an attached apex, six being a shelf-like cusp with an attached apex, and seven being a fully formed cusp with a free apex, separate from the protocone (Hillson, 2002). Therefore, an ASUDAS score of zero represents complete absence of the trait, while a score of seven represents the fully formed, unattached cuspal version of the trait. ASUDAS scores one through six represent intermediate Carabelli expressions. My research employs this eight-point graded scale. In addition, my study expands on nonmetric measurements of the cusp to include, whenever possible, measured area data for cuspal forms of the trait.

Evolutionary significance

New cusps arise for many reasons. They may become common in a population to improve food-processing techniques or to increase occlusal surface. Many studies have found that Carabelli presence is indeed associated with increased tooth area (Korenhof, 1960; Keene, 1968; Lombardi, 1975; Reid et al., 1991, 1992; Kondo and Townsend, 2006; Harris, 2007), although some earlier research suggested that no such relationship exists (Garn et al., 1966a). Given selective pressures and specific developmental and genetic circumstances, an accessory cusp such as Carabelli's could become a fixed trait in a population or species. It could be argued that Carabelli's cusp is not being selected for, since it has never become fixed in a human population. Hanihara (2008) found that nonmetric dental variation as a whole has not been under selective pressure in the recent past. Alternatively, it could be argued that the trait is undergoing selection, since it has persisted in modern human populations and is observable in both hominids and primates. Studies of Carabelli frequency over time have yielded mixed results. Brabant (1971) found an increase in trait frequency from Neolithic to modern European populations, while Hsu et al. (1997) noted a decrease in trait frequency from aboriginal to modern Chinese populations. However, the purpose of this study is not to conclude or speculate as to why Carabelli's cusp is seen in varying frequencies in different populations. It instead focuses on studying and demonstrating under what circumstances an accessory cusp is likely to develop.

Salazar-Ciudad & Jernvall's patterning cascade model

Many genes that affect tooth development have been identified, however none of them have been shown to code for the development of specific cusps (Salazar-Ciudad and Jernvall, 2002). Under this assumption, there is no "Carabelli gene" that turns on to signal Carabelli formation in some individuals. Instead, the many genes that are responsible for tooth formation determine whether or not and to what extent a Carabelli's cusp will form.

Salazar-Ciudad and Jernvall (2002) described a model that accounts for such tooth formation and differentiation. Through small changes in the model's principles, tooth morphology can be drastically altered. By tweaking the model's parameters, Salazar-Ciudad and Jernvall (2002) were able to accurately predict cusp formation in mice and voles.

The model specifically predicts tooth development from the cap stage to the early bud stage and does not include mineralization (Salazar-Ciudad and Jernvall, 2002). Despite this, since the model accurately predicted not only the forms of mice and vole teeth, but also the paths they took to develop, it may be useful as an overall predictor of development (Salazar-Ciudad and Jernvall, 2002). It can be justifiably used as a general model of cusp development, and it can be applied to human tooth morphology and the development of cusps, including accessory cusps such as Carabelli.

The cap stage begins with the initial growth of epithelium into the mesenchyme tissue beneath it (Salazar-Ciudad and Jernvall, 2002). This produces the primary enamel knot and is the future location of the tallest cusp. Enamel knots are bundles of undifferentiating epithelial cells that mark the tips of cusps and control further epithelial proliferation through more than ten different signaling molecules (Thesleff et al., 2001). After the formation of an enamel knot, activators and inhibitors control tissue proliferation. Activators and inhibitors are types of signaling molecules whose concentrations and diffusions ultimately determine tooth morphology. Activators cause epithelium to proliferate and epithelial knots to form, while inhibitors block new enamel knots from forming and promote growth (Salazar-Ciudad and Jernvall, 2002). Therefore, a new enamel knot cannot form until it is out of the effective range of the inhibitory molecules of a preexisting knot. Only then can activators promote tissue differentiation and form a new enamel knot. The three-dimensionality of tissue created by preexisting enamel knots ultimately affects new enamel knots, since previous morphology determines the diffusion of signaling molecules as well as the space allowed for a new knot to form (Salazar-Ciudad and Jernvall, 2002). A new enamel knot cannot form until it escapes the inhibitory signaling molecules released by a preexisting knot. We would expect such knots to form when preexisting knots are spaced closer together relative to the crown area, allowing for new cusps to form prior to cessation of growth (Fig. 4).

Tooth structure and development are implicitly related, a condition that the authors refer to as “morphodynamic” (Salazar-Ciudad and Jernvall, 2002). This developmental model has also been referred to as “patterning cascade,” a term which describes the relationship of cusp development to a cascade of developmental events (Jernvall and Jung, 2000). Small changes in the model’s parameters can therefore have a profound affect on tooth morphology. Small changes in such parameters can lead to big changes in morphology, such as the formation of a new cusp. Since the model was developed to predict mice and vole tooth development, it is interesting to investigate the model's application to human populations. It demonstrates the conservative nature of developmental genes in mammals. Small genetic changes can lead to a high range of phenotypic diversity (Salazar-Ciudad and Jernvall, 2002).

Variation between populations can arise due to changes in the parameters of the model (Salazar-Ciudad and Jernvall, 2002). This study tests if the predictions set forth by the patterning cascade model apply to the expression of Carabelli's cusp in modern adult humans. It will test if variation between human populations and between the sexes in Carabelli expression can be explained by the model's predictions and parameters. In this case, we would expect Carabelli's trait to increase in size and development as intercusp spacing, relative to tooth area, decreases.

For a more tangible example of the model's predictions, imagine two teeth with exactly the same occlusal area. The main cusps on Tooth A are spaced closely together. Tooth B has more widely spaced main cusps, near the peripheral edges of the crown. During development, the model would predict that Tooth A, the tooth with smaller relative intercusp distances, would be more likely to form an accessory cusp than Tooth B. During development, as Tooth A's epithelium proliferates outward farther from enamel knots, there would be a greater chance that a new enamel knot could escape the inhibitory signalers of closely-spaced preexisting knots, thereby forming a new cusp. Tooth B's widely spaced cusps would likely prevent an accessory cusp from forming prior to cessation of development.

This example sums up the predictions of the model as they apply to the development of Carabelli's cusp. Based on the model, we would expect teeth that have larger crown areas relative to smaller intercusp distances to display Carabelli more frequently and in larger and more developed forms. These predictions are summarized in Figure 5.

The populations

Rene Menagaz-Bock donated the Gullah and Seminole dental casts to the Ohio State University Bioarchaeology Laboratory. The Gullah sample comes from a population of African Americans from Florida, collected between 1960 and 1970. The Seminole sample comes from of a population of Native Americans, also collected between 1960 and 1970. The Seminole sample did not include geographic information. I chose these populations because they each consist of only one ethnic group. Many of the other cast sets available to me were from populations with mixed ethnic backgrounds. In addition, if previous research about Carabelli frequency for these two populations is correct (African- and European-descended populations should have higher trait prevalence than Asian-descended populations), this study should be able to determine if these differences are explainable by the patterning cascade model of development.

When assessing human variation and phenotypes, both genetics and environmental effects must be considered. Environment influences phenotype during fetal development and through growth into adulthood. Although crown and root development are influenced by environmental factors, Scott and Turner (1997) stated that no research has found that environment significantly affects nonmetric morphological crown traits. However, Kolakowski et al. (1980) found a significant environmental effect on Carabelli's trait. Carabelli's cusp should develop and be expressed under the same developmental parameters regardless of environment. The different environments in which the study populations developed may have had some influence on the extent of Carabelli expression, but they did not influence the processes by which the cusp develops. It is therefore acceptable to assume that only genetic and developmental factors are responsible for Carabelli development in the studied populations, although environmental effects may limit development and lead to decreased Carabelli expression.

Previous anthropological research

Modern anthropological studies have examined Carabelli's trait in an attempt to establish its frequencies in different human populations. It is widely believed to be a common and defining trait of the 'Caucasoid dental complex' (Mayhall et al., 1982) and largely absent from the 'mongoloid dental complex' of Asians and Native Americans (Hanihara, 1969). Hassanali (1982) found that Carabelli occurs in higher frequencies in modern Africans than Asians. Scott (1980) found that Carabelli's trait is approximately twice as prevalent in populations of European descent than in Pacific Island populations. Meredith and Hixon (1954) found an 84% frequency of the cusp in upper permanent first molars for individuals of northwest European descent. Kolakowski et al. (1980) stated that its presence ranges from 7-13% in Asian populations (Suzuki and Sakai, 1957) to 80% in Caucasian populations (Alvesalo et al., 1975). Other studies have found that, compared to African or Asian populations, Carabelli's cusp is a marker of European ancestry (Whittaker and MacDonald, 1989; Rhine, 1990). Turner and Hawkey (1998) found that the trait is more prevalent in African populations than any other population, including Europeans.

Other studies have looked at Carabelli sex differences with varying results. Harris (2007) found no Carabelli sexual dimorphism in a sample of 300 North American whites. Scott (1980) found no sexual dimorphism of the trait in south Australian whites. Other studies have agreed with these findings (Garn et al., 1966b; Turner, 1969, Alvesalso et al., 1975; Kieser, 1978; Hassanali, 1982; Saunders and Mayhall, 1982; Thomas et al., 1986). Tsai et al. (1996), on the other hand, found that Carabelli is sexually dimorphic in a population of Taiwan aborigines. Brabant (1971) and Kondo and Townsend (2006) also found that males displayed greater Carabelli development. Other studies found the trait to be sexually dimorphic as well (Goose and Lee, 1971; Kaul and Prakash, 1981; Kieser and Preston, 1981). Noss et al. (1983) state that inadequate sample sizes are to blame for studies that find insignificant sex differences. Due to these mixed results on the sexual dimorphism of the cusp, and possible variability of sexual dimorphism in different populations, this study will examine males and females separately when appropriate. Toma et al. (2007) suggest that male samples have larger tooth size and between-group difference than female samples, and therefore express salient features of each sample more clearly. This could explain differences in trait expression observed between males and females of each population.

If between-population differences are to be expected, they should be explainable by the model if one population tends to differ from another in overall tooth size, cusp spacing, or both. Alternatively, populations with similar-sized teeth and cusp spacing could still differ from one another in Carabelli expression if small developmental parameters, for instance inhibitory signalers or threshold differences, are variable between the populations. If the patterning cascade model can account for Carabelli development, in terms of cusp spacing and tooth size, then we should expect to see differences between the sexes in Carabelli expression. There is a smaller degree of sexual dimorphism in intercusp spacing than in buccolingual and mesiodistal measurements (Townsend, Richards, and Hughes, 2003). In other words, compared to female teeth, male teeth should increase in overall size at a faster rate than they increase in intercusp spacing. Following the model's predictions, we should therefore expect to see greater Carabelli expression in male teeth. We should hence also expect to observe greater Carabelli expression in larger teeth. Kondo and Townsend (2006) support this prediction.

Harris (2007) found that Carabelli's cusp is associated with "factors affecting overall crown size, including the spacing among cusp tips that depends on cusp sizes" (2007:245). These findings agree with the predictions of the patterning cascade model.

Testing the model in a Dayton population

Previous research by Hunter et al. (2010) studied the applicability of the patterning cascade model in a population of Dayton orthodontic patients. The study examined the relationship between Carabelli expression and cusp spacing in a sample of 376 upper first molars (Hunter et al., 2010). It measured Carabelli expression both qualitatively using ASUDAS as well as quantitatively using Carabelli area. The methods that were used to measure Carabelli development are the same used in my study. The prediction is that Carabelli development will increase as intercusp spacing, relative to tooth size, decreases. This fits the model's predictions: when cusps become more closely spaced, the "risk" of expressing a more developed Carabelli's cusp increases. These predictions are summed up in Figure 5. The study by Hunter et al. (2010) found that Carabelli's cusp is more likely to be present and increase in development and size as intercusp spacing relative to overall tooth size decreases. These findings support the predictions made by Salazar-Ciudad and Jernvall's patterning cascade model. My research expands on this study to test if these same predictions are true across populations and sexes.

RESEARCH QUESTIONS

The following is a list of research questions that I will address in the results and discussion sections:

1. Are there differences in Carabelli expression between the populations?

2. Are there differences in Carabelli expression between the sexes?

3. Are there differences in intercusp spacing between the populations or between the sexes, and if so, are they in the direction predicted by the model?

4. Is Carabelli expression associated with relative intercusp spacing in populations and sexes, and can differences in Carabelli expression between populations and sexes be explained by differences in relative intercusp spacing?

5. Can Carabelli development be attributed to the geometric spacing of certain cusps?

METHODS

The teeth measured in this study come from dental casts that were collected and stored in the Ohio State University Bioarchaeology Research Laboratory. Casts can be considered accurate representations of the original dentition, since there is only a 0.1% difference in measurements between casts and their originals (Hunter and Priest, 1960). Permanent maxillary first molars comprised the entirety of teeth surveyed in this study. In addition, I only measured teeth whose total crown extent could be easily defined and whose cusps and lingual aspect of the protocone (location of Carabelli's cusp) were intact. Each cast has a unique label number. Information that is catalogued in the Bioarchaeology Laboratory contains data on each population, including sex for the Gullah and Seminole populations. In the New York population, I used first names that are written on the casts to classify sex. When the first name could be considered gender-ambiguous, I assigned a classification of “unknown” for sex.

The total sample includes 209 teeth from the Gullah population, 183 teeth from the Seminole population, and 90 teeth from the New York population. In the Gullah sample, 83 teeth are from males, 114 are from females, and 12 are unclassified. In the Seminole sample, 86 teeth are from males and 97 are from females. The New York population contains 17 male teeth, 44 female teeth, and 29 unclassified teeth. Refer to Table 1 for a further breakdown of the data.

I used a Hirox digital microscope, model number KH-7700, to take measurements and pictures of the teeth. In order to obtain the most consistent measurements as possible, the cusps needed to be oriented in the same manner, with their cusps as level as possible. I accomplished this by one of two methods: the cast was either suspended by a clip stand or positioned with Play-Doh. The clip stand provided more stability and proved easier to orient, and I therefore used it for the majority of measurements. Casts that were too heavy or bulky to be supported by the clip stand were oriented with Play-Doh.

Prior to measurement, I graded each tooth’s Carabelli development using the ASUDAS eight-point scale (Fig. 3). I then marked the tip of each cusp with a pencil. I also marked the periphery of cuspal forms of Carabelli if I had difficulty discerning its extent under the microscope. I measured the teeth with a low lens adapter in a 15 x 24 millimeter field of view. I calibrated the microscope by zooming in and bringing into focus the pencil mark on the tip of the paracone before returning to the 15 x 24 millimeter field of view. I took two-dimensional measurements of each tooth and recorded data for maximum mesiodistal distance, maximum buccolingual distance, total tooth area, six intercusp distance measurements between the four main cusps, Carabelli area if present, and Carabelli-protocone intercusp distance if Carabelli was present (Fig. 6). Intercusp distances, mesiodistal, and buccolingual measurements were simple line segment measurements. Total tooth area and Carabelli area were area measurements. These measurements involved creating closely spaced points around the entire periphery of either the crown or Carabelli's cusp and then generating the area encompassed by these points. The measurements provide an estimate of crown area and the area of Carabelli's cusp.

Treatment of Carabelli's cusp as a variable

Carabelli's cusp can be analyzed in two ways: by only examining teeth with measurable, cuspal forms of the trait (ASUDAS = 5 – 7) or by studying all teeth, using ASUDAS categories to classify Carabelli development. The ASUDAS variable itself can be looked at in two different ways. First, it can be considered an ordinal variable. The ASUDAS system classifies Carabelli's cusp on an eight-point scale from 0 to 7, with each subsequent number corresponding to greater Carabelli development. It can also be considered a "quasi-continuous" variable. Quasi-continuous variables have visible and underlying scales corresponding to the presence and absence of a trait, respectively (Scott and Turner, 1997). The underlying range is continuous, representing a range of genes that do not express the trait until a threshold is crossed, at which point the trait is present and classifiable in discontinuous intervals (Scott and Turner, 1997). A range of different genotypes may therefore lie below the threshold, all of them leading to an absence of trait expression. Carabelli's trait can be classified as quasi-continuous (Harris, 1977), and I will therefore examine ASUDAS classification as a continuous variable as well as an ordinal variable.

RESULTS

I used IBM® SPSS® Statistics Version 19 to process almost all of the statistical analyses for this project. The exception is the proportional odds logistic regression test with ASUDAS as a response variable and sex, population, and relative intercusp average as predictor variables. John Hunter ran this test with the statistical program R.

Measurement error

The Hunter et al. (2010) study assessed my measurement error for the study of Carabelli expression in a Dayton population. I will cite those numbers as measurement error for this study as well, since the types of measurements that I conducted were the same in both studies. Measurement error is relative and expressed as a percentage of variation within and among individuals. This was assessed through Model II ANOVA. Percent measurement area was moderately high for intercusp distances (12-32%) and lower for tooth area (10%) and Carabelli area (4%). The error for intercusp distances may be due to their small dimensions, subjectivity in marking the cusp tips, or the repeatability of orienting the teeth. Measurement error will not be factored into the results since this may increase the probability of obtaining a false positive result, and I will instead offset the effects of this measurement error through a large sample size (Hunter et al., 2010). Since measurement error should be random, it should obstruct any correlations in the data or create any additional correlations. This is reinforced by the large sample size, which decreases the power of random measurement error.

Preliminary analyses

In order to obtain an estimation of cusp spacing, I used a measurement termed "intercusp average." The is simply the mean of the six intercusp measurements between the four main cusps: paracone to protocone, paracone to metacone, metacone to protocone, paracone to hypocone, protocone to hypocone, and metacone to hypocone. This gives an estimation of the average cusp spacing of each individual tooth. A smaller number means that the cusps are more closely spaced, and a larger number means that the cusps are farther apart. In order to compare a tooth's cusp spacing to those of other teeth, I used a measurement termed "relative intercusp average." This measurement divides the intercusp average by the square root of the total area of the tooth. Relative intercusp average can be used to compare teeth, since it is a relative measure, not an absolute one like intercusp average. I used the square root of the total area in the calculation, as opposed to a raw measurement of total area, in order to keep intercusp average and area in the same dimension. Intercusp average and the square root of the total area are both measured in millimeters, while total area is simply measured in square millimeters.

Preliminary analyses reveal basic trends in the data. Figure 7 is a box-and-whisker plot that shows decreasing relative intercusp average with increasing Carabelli development. Carabelli development is measured in this figure as "Absent" (ASUDAS = 0), "Slight" (ASUDAS = 1 – 6), or "Present" (ASUDAS = 7). "Absent" is therefore indicative of complete Carabelli absence, while "Present" denotes full cuspal development. Figure 8 depicts the differences between present Carabelli and absent Carabelli teeth in their relationships to tooth size and cusp spacing. Present Carabelli teeth have smaller intercusp averages relative to tooth size. Present Carabelli teeth tend to fall below the regression line, while absent Carabelli teeth tend to fall above it. The mean residual value for present Carabelli teeth is -0.333723 and below the regression line while the mean residual value for absent Carabelli teeth is 0.069961 and above the regression line. These findings support the predictions of Figure 5. Figure 9 and Figure 10 show that decreasing relative intercusp average is associated with greater Carabelli expression. Figure 9 includes all teeth and shows how relative intercusp average decreases as Carabelli expression, measured as ASUDAS, increases. Figure 10, which includes only teeth with measureable Carabelli's cusps, shows the decrease in relative intercusp average as Carabelli expression, measured as square root Carabelli area, increases.

A Pearson Correlation shows that increasing ASUDAS is associated with increasing tooth area ($P = 0.005$). A t-test also shows that teeth with present Carabelli's cusps (ASUDAS = 7) have significantly larger tooth areas ($t(73) = 2.390$, $P = 0.019$) than teeth lacking the trait entirely (ASUDAS = 0).

Differences between the populations?

In my population and sex comparison analyses, I only included males and females from the Gullah and Seminole populations. I made this decision because I felt it was the best way to study the effects of cusp spacing, population, and sex on Carabelli development. The New York sample was small and included few male teeth and many teeth whose sex could not be determined. There were more males in both the Gullah and Seminole samples, and the Seminole sample included no teeth whose sex was unknown while the Gullah sample included only twelve teeth of unknown sex. Considering only teeth that could be identified as male or female, the Gullah population contained 42.13% male teeth and the Seminole population had 46.99% male teeth, while the New York population was made up of only 27.87% male teeth. Analyses that utilized sex and population only included teeth that had been identified as male or female from the Gullah and Seminole populations. For the rest of the results section, it can be assumed that only male and female teeth from the Gullah and Seminole populations are being considered, unless otherwise noted.

When all the samples from the Seminole and Gullah populations are pooled, including teeth with unknown sex, *t*-tests show that both Carabelli area ($t(60.251) = 2.875$, $P = 0.006$) and relative Carabelli area ($t(60.831) = 2.972$, $P = 0.004$) are significantly larger in the Gullah population. Relative Carabelli area is a measurement of the size of Carabelli's cusp compared to the entire area of the tooth, including Carabelli's cusp, and it is measured as: (absolute Carabelli area) / (total area). When only males are considered, Gullah males still have both significantly larger Carabelli area ($t(31) = 2.183$, $P = 0.037$) and relative Carabelli area ($t(23.093) = 2.674$, $P = 0.014$) values compared to Seminole males. The same cannot be said of the female sample. Although Gullah females had both larger mean Carabelli areas and relative Carabelli areas, the differences between female Gullah and Seminoles in Carabelli area ($t(27) = 1.307$, $P = 0.202$) and relative Carabelli area ($t(27) = 0.833$, $P = 0.412$) are not significant.

When assessed as a continuous variable using *t*-tests, ASUDAS values are not significantly different between the populations when both sexes and sex unknown are pooled ($t(390) = 1.632$, $P = 0.104$) or when males ($t(167) = 1.125$, $P = 0.262$) or females ($t(209) = 0.929$, $P = 0.354$) are considered separately. In each case, the Gullah population has a larger mean, but it is not significant.

Mann Whitney *U*-tests show that there is not a significant difference in ASUDAS distributions between the Gullah and Seminole in the pooled sample ($P = 0.114$), males only ($P = 0.263$), or females only ($P = 0.362$).

It is also worth noting that, although there are seventeen fully formed (ASUDAS = 7) Carabelli's cusps in the Gullah population, there are none in the Seminole population. Both of these counts differ greatly from their expected counts (Table 2).

There are several other methods to examine cusp frequency by categorizing the trait as present or absent based on different criteria. Cusp presence versus cusp absence could be viewed as complete absence versus any form of the trait (ASUDAS = 0 versus ASUDAS = 1 – 7), absence, furrows, and pits versus grooves and cuspal forms (ASUDAS = 0 – 2 versus ASUDAS = 3 – 7) or non-cuspal forms of the trait versus cuspal forms (ASUDAS = 0 – 4 versus ASUDAS = 5 – 7). I compared the entire Gullah sample, including unknown sex, to the entire Seminole sample for each classification. For the first criterion (ASUDAS = 0 versus ASUDAS = 1 – 7), the Gullah sample has 91.4% presence versus 88.5% presence in the Seminole sample. This difference is not significant ($t(390) = 0.943$, $P = 0.346$). For the second criterion (ASUDAS = 0 – 2 versus ASUDAS = 3 – 7), the Gullah sample has 52.2% presence while the Seminole sample has 43.7% presence. This result is marginally significant ($t(390) = 1.670$, $P = 0.096$). For the third criterion (ASUDAS = 0 – 4 versus ASUDAS = 5 – 7), the Gullah sample has 17.2% presence and the Seminole sample has 15.3% presence. This difference is not significant ($t(390) = 0.513$, $P = 0.608$).

Differences between the sexes?

T-tests show that there is no significant difference between males of both populations and females of both populations in Carabelli area ($t(60) = 0.225$, $P = 0.823$) and relative Carabelli area ($t(60) = -0.612$, $P = 0.543$). There is no significance when Carabelli area ($t(32) = 0.741$, $P = 0.464$) and relative Carabelli area ($t(32) = 0.409$, $P = 0.685$) are compared for only Gullah males and females, and also when Carabelli area ($t(26) = -0.137$, $P = 0.892$) and relative Carabelli area ($t(26) = -1.290$, $P = 0.208$) are considered between only Seminole males and females.

ASUDAS, assessed as a continuous variable, is marginally significantly greater in males when both populations are pooled ($t(378) = 1.914$, $P = 0.056$). However, when the populations are divided into Gullah ($t(195) = 1.498$, $P = 0.136$) and Seminole ($t(181) = 1.300$, $P = 0.195$), this marginal significance breaks down. Males have larger mean ASUDAS values than females in both of these cases, but neither is significant.

A Mann Whitney *U*-test of the including both populations shows a significant difference in ASUDAS distributions between the sexes ($P = 0.028$). ASUDAS distribution shows only marginally significant difference when only Gullah males and females are compared ($P = 0.081$). The distribution is not significant when only Seminole males and females are compared ($P = 0.135$).

Differences in intercusp spacing?

Between populations, relative intercusp average is significantly greater in the Seminole population when both sexes are included ($t(378) = -5.024$, $P < 0.0005$). When only males are considered, relative intercusp average is only marginally significantly greater in the Seminole population ($t(167) = -1.684$, $P = 0.094$). When only females are considered, relative intercusp average is significantly greater in the Seminole population ($t(209) = -5.232$, $P < 0.0005$).

Between the sexes, relative intercusp average is not significantly different when both populations are included ($t(378) = 0.206$, $P = 0.837$). When the populations are divided, relative intercusp average is not significantly different between Gullah males and females ($t(195) =$

1.551, $P = 0.123$), but it is marginally significantly greater in females than males in the Seminole population ($t(181) = -1.693$, $P = 0.092$).

Which factors affect Carabelli expression?

I used a general linear model to analyze the effects of relative intercusp average, sex, and population on square root Carabelli area. The model revealed no main effects of sex ($F(1, 57) = 0.456$, $P = 0.502$) and population ($F(1, 57) = 3.089$, $P = 0.084$), and no interaction between sex and population ($F(1, 57) = 3.474$, $P = 0.067$). The model did show significant effects of relative intercusp average on square root Carabelli area ($F(1, 57) = 74.580$, $P = 0.000$).

I employed a multiple linear regression test to examine the effects of relative intercusp average, sex, and population on Carabelli development, expressed as ASUDAS in the form of a continuous variable. The overall model was significant (adjusted R square = 0.017, $F(3, 376) = 3.221$, $P = 0.023$). Both relative intercusp average (Beta = -0.104, $P = 0.050$) and sex (Beta = 0.101, $P = 0.047$) were significant predictor variables, while population (Beta = -0.048, $P = 0.366$) was not.

I assessed ASUDAS as an ordinal variable using proportional odds logistic regression. In this model, ASUDAS, as a measurement of Carabelli expression, was the response variable, and sex, population, and relative intercusp average were predictor variables. Results can be viewed in Table 3. The model was significant at $P < 0.005$. Model significance was assessed as a likelihood ratio test between the model's alternative hypothesis and null hypothesis.

Ordered logistic regression assumes common slopes for the transitions between each threshold (ASUDAS = 0 to ASUDAS = 1, ASUDAS = 1 to ASUDAS = 2, ASUDAS = 2 to ASUDAS = 3, etc.). The analysis calculated intercepts for each transition and slope coefficients for each predictor variable. These coefficients are log-odds ratios, and they can be exponentiated to give odds ratios for the outcome of Carabelli development, expressed as ASUDAS (Agresti, 2007).

The coefficient for population shows that population is negatively related to ASUDAS. Following my coding scheme, this result means that the Gullah population has greater Carabelli expression than the Seminole population. When exponentiated, the coefficient becomes an odds ratio of 0.822255. Since population is in units of one, this odds ratio can be interpreted as is. Population is coded for 1 = Gullah and 2 = Seminole, so being a tooth in the Seminole population (population = 2) decreases the likelihood of Carabelli expression at each ASUDAS transition by a factor of about 0.8 to 1.0. This ratio is fairly close to 1.0 to 1.0.

The coefficient for sex shows that it is positively correlated with ASUDAS. By my coding method, this means that males have greater Carabelli expression than females. Sex is also in units of one, so the odds ratio, found by exponentiating the coefficient, can also be interpreted as is at 1.534353. Considering the coding, this means that being sex two (male) over sex one (female) increases the likelihood of Carabelli expression by a ratio of about 1.5 to 1.0.

The coefficient for relative intercusp average reveals that it is negatively related to ASUDAS. This is expected; as relative intercusp spacing decreases, Carabelli expression increases. Although relative intercusp average can theoretically range from zero to one, the range of my data for relative intercusp average is approximately 0.5 to 0.7. This is an approximate range of 0.2. Therefore, in order to best interpret the results, the coefficient should be scaled by 0.2 or less before being converted to an odds ratio to yield interpretable results. Scaling the coefficient by 0.2 and converting it to an odds ratio gives a ratio of 0.389338 to 1.0. This means that teeth with the smallest relative intercusp averages are about two and a half times more likely than teeth with the largest relative intercusp averages to have higher ASUDAS scores. Scaling the coefficient by 0.1 and then converting it to an odds ratio yields a ratio of 0.623969 to 1.0. This tells us that teeth with the smallest relative intercusp averages are approximately 1.6 times more likely to have higher ASUDAS scores than teeth in the middle of relative intercusp averages.

Carabelli development and cusp geometry

A partial correlation reveals that only certain intercusp distances significantly correlate with Carabelli expression. The control variables that were held constant in this analysis were total tooth area, sex, and population. The test shows the correlations between each of the six main intercusp measurements and Carabelli development, measured as ASUDAS (Table 4). The paracone-protocone and metacone-protocone distances significantly negatively correlate with increasing ASUDAS Carabelli expression (Fig. 11). As these two intercusp distances decrease, ASUDAS increases.

DISCUSSION

My analysis shows that increased Carabelli development is related to decreased relative intercusp spacing. These findings match the predictions of Jernvall and Jung (2000) and support the findings of Hunter et al. (2010). As cusp spacing becomes smaller, relative to overall crown size, we see a greater risk of developing a new cusp, in this case, Carabelli.

My findings also indicate that greater Carabelli expression is related to increased tooth size. These results are consistent with previous research. Kondo and Townsend (2006) demonstrated that, when growth is prolonged during development, dental epithelium is given increased time to fold, leading to cuspal Carabelli development and larger crown size. This is not to say that one causes the other. Rather, as development is prolonged and epithelium continues to fold, tooth size increases, as do the odds of a new enamel knot escaping inhibitory signalers and forming Carabelli's cusp.

The population-level results are difficult to interpret. While the Gullah population displays significantly greater Carabelli area when both sexes are considered and when males of the two populations are compared, the same is not true when only females are compared (although Gullah females had greater mean Carabelli area than Seminole females, despite the lack of significance). When Carabelli development is assessed through ASUDAS, both as a continuous and ordinal variable, it is not significantly different between the populations. However, ASUDAS mean is significantly greater in the Gullah population, regardless of if it is analyzed as a whole or if it is broken up into male/female categories. Relative intercusp average is significantly smaller in the Gullah population when assessed for both sexes combined as well as for females only. This fits with the significantly greater Carabelli area in the combined sample. However, Gullah males display significantly greater Carabelli development than Seminole males, although relative intercusp average is not significantly different between the two. For females, the difference in Carabelli area is not significantly different between the populations, although relative intercusp average is. Despite this, both the male and female groups follow the predicted trend regardless of significance; Gullah populations in both cases have higher mean Carabelli areas and lower mean relative intercusp averages. It is difficult to draw conclusions based on this evidence about population-level differences in Carabelli expression or in the effects that population has on Carabelli expression.

The results show greater Carabelli expression in the Gullah population. Although not always significant, expected trends are observed. This finding agrees with previously mentioned studies that have demonstrated the greater prevalence of the trait in African-descended populations and lesser prevalence in Asian-descended populations. This trend can be observed specifically in the cuspal (ASUDAS = 5 – 7) forms of the trait. The Gullah population displays more developed and larger forms of the cuspal form of the trait. In addition, I saw no fully formed Carabelli cusps in the Seminole population, while I observed seventeen in the Gullah.

Despite the fact that the cuspal form of Carabelli is more prevalent in the Gullah population, this study shows that it cannot always be considered a significant marker of particular populations. Turner and Hawkey (1998) reached a similar conclusion. They found that Carabelli's trait does not follow any geographic or genetic patterns, and that expression of the cusp is not a reliable marker of European populations (Turner and Hawkey, 1998).

In the analyses between the sexes, Carabelli area is marginally significantly greater in males in the pooled sample. When broken up into the different populations, marginally significant differences between the sexes in Carabelli area fall apart. With both sexes pooled, ASUDAS is marginally significantly greater for males, but this marginal significance is also lost when divided into populations. Mann Whitney *U*-tests also show a significant difference in ASUDAS distribution between the sexes, which also falls apart into marginal significance when only the Gullah are considered and non-significance when only the Seminole are considered. These results suggest that sex differences may exist. If so, any significance or marginal significance may fall apart when the populations are considered separately due to smaller sample size. It is worth noting the previously mentioned opinion of Noss et al. (1983) that small sample sizes can be blamed for studies that find insignificant sex differences. Although the sample sizes are not small, evaluating sex differences separately by population decreases the significance of my findings. Smaller sample sizes have less power because of the relationship of the magnitude of the sample to a similar degree of sex differences present in both the large pooled sample and the smaller subsamples. It is possible that increasing the sample size could make these findings significant. Of greatest importance may be my finding that any sex differences apparently cannot be attributed to significant differences in relative intercusp average between the males and females, since *t*-tests showed no significant differences between the sexes. This hints that sex, as a variable, may have a separate effect from relative intercusp average on Carabelli expression.

Analyses that take into account multiple variables including Carabelli development (measured as ASUDAS or square root Carabelli area), relative intercusp average, sex, and population allow for a more complete analysis of the results by creating models of interactions and correlations among the variables. Testing Carabelli development as square root Carabelli area shows that relative intercusp spacing is the only variable that significantly correlates with Carabelli size, while population and sex do not. When Carabelli development is assessed as ASUDAS, which itself is assessed as a continuous variable, both relative intercusp spacing and sex are significant variables, while population is not. My analysis of ASUDAS as an ordinal variable yielded similar results: relative intercusp average and sex are more greatly correlated with Carabelli expression than population is.

Viewing the results of these tests together, I infer that relative cusp spacing is the main contributor to Carabelli development. Relative intercusp average was the only variable to significantly correlate with Carabelli development when only cuspal forms were considered. This was true of the model with ASUDAS as an ordinal variable as well. The risk of developing

Carabelli's cusp is most greatly determined by the spacing of preexisting enamel knots epithelium is proliferates during odontogenesis.

In addition to my findings on the affect of relative cusp spacing on Carabelli development, the tests of ASUDAS as both a continuous and ordinal variable show that sex is also a significant factor in Carabelli development when measured as ASUDAS. In addition, *t*-tests showed no significant differences between the sexes in relative intercusp average. Differences between the sexes in Carabelli expression can therefore not be attributed solely to relative intercusp average. The statistical tests themselves cannot suggest why this may be the case. However, if sex is also a contributor to Carabelli development, it could be due to differences between males and females in developmental parameters explainable by the model. Small changes to these developmental parameters could lead to large changes in morphology. For instance, differences in signaler molecule concentrations could lead to differences in the timing of enamel knot initiation. Suppose that enamel knots in female teeth produce inhibitory molecules in a concentration that is, on average, greater than enamel knots in male teeth. In this case, females' enamel knots would have larger inhibitory zones than the enamel knots of males. Greater spacing would be required for new female enamel knots to escape these larger inhibition zones, and therefore there would be greater distance between the cusps. Females would have cusps that are more widely spaced than those of males, and they would have larger relative intercusp averages. Under these circumstances, we would expect males, if they have smaller relative intercusp averages, to display greater Carabelli expression. Differences in the timing of development could also explain Carabelli expression. The timing of the apoptosis of the enamel knot cells is one possible parameter that could affect morphology (Tucker and Sharpe, 1999). Tucker and Sharpe (1999) suggest that the timing of enamel knot apoptosis "could lead to differences in cusp shape and size" (78:830). Early cell death of the enamel knot cells would stop the dispersion of signaler molecules and allow more closely spaced cusps than later cell death. Small changes in developmental parameters between the sexes could explain why sex appears to significantly correlate with Carabelli expression measured as ASUDAS.

The results of the partial correlation analysis, from a geometrical perspective, fit the predictions of the patterning cascade model. The "risk" of Carabelli development ultimately lies on the position of the protocone. If the Carabelli enamel knot cannot escape from inhibition by the protocone enamel knot, it will not form. As the protocone becomes more closely spaced to the paracone and metacone, Carabelli "risk" increases.

If Carabelli "risk" ultimately lies with the position of the protocone, one may wonder why protocone-hypocone intercusp distance does not also significantly correlate with ASUDAS. This can be explained by the fact that the hypocone is typically the smallest and shortest and therefore likely the final major cusp to form. Since the cuspal form of Carabelli is present at the enamel-dentine junction in humans (Sakai and Hanamura, 1971; Sasaki, 1997), the cusp is subject, like the other cusps, to formation by the folding of enamel epithelium and its presence can be explained by the mechanisms of the patterning cascade model. It is therefore not affected by the folding of the hypocone. By the time it forms, the protocone has already formed, and therefore the protocone-hypocone intercusp distance does no affect the odds of Carabelli formation. Despite the fact that no significant correlation exists in the protocone-hypocone intercusp distance, hypocone development correlates significantly with Carabelli development (Scott, 1979). This could be due to other developmental factors under which the two cusps are more likely to form, such as overall cusp spacing, and may not depend on one specific intercusp distance such as the protocone-hypocone intercusp distance.

Homoplasy and population affinity studies

Anthropological studies have frequently used independent nonmetric dental traits as a means of measuring affinity between populations (Turner and Hawkey, 1998). This research calls into question the validity of such methods.

Previously mentioned research has demonstrated differing frequencies of Carabelli's trait between populations, mainly the greater presence of the trait in European- and African-descended populations than in Asian-descended populations. Such studies rely on correlating one specific dental morphological trait with particular populations. However, other studies have demonstrated relationships between Carabelli's cusp and other dental traits. Carabelli's cusp is correlated with both the protostylid in lower molars (Scott, 1978) and the hypocone in upper molars (Scott, 1979). Jernvall and Jung (2000) state that "the patterning cascade mode of development may increase the likelihood of homoplasy and flickering" (43:186). My findings agree with this hypothesis and support the findings of Hunter et al. (2010) that Carabelli and other dental traits that correlate with it are related through changes in the same developmental parameters. Changes in these parameters can lead to not only changes in Carabelli expression, but also changes in expression of other correlated dental traits. In this type of model, we expect the probability of homoplasy between Carabelli and these other traits to be high (Hunter et al., 2010). This can lead to problems when assessing phylogenetic relationships while assuming independence of Carabelli or other traits (Kangas et al., 2004). Non-molar traits should be considered as well. Traditional beliefs about population affinity markers, such as a higher frequency of Carabelli in European-descended populations and a higher frequency of incisor shoveling in Asian-descended populations, may not be correct. Tsia et al. (1996) found that incisor shoveling presence increases the odds of Carabelli by three times. Nonmetric traits, when used as population affinity markers, are best assessed together, not independently.

CONCLUSION

My study lends support to previous evidence that Carabelli's cusp is subject to development as described in Salazar-Ciudad and Jernvall's (2002) patterning cascade model. Teeth whose main cusps are more closely spaced are more likely to develop Carabelli's cusp. In addition, teeth are more likely to have a larger, more developed Carabelli's cusp when tooth growth is prolonged. Considering the model and these findings, we would expect a tooth with small intercusp distances and large tooth area to be most likely form a large, fully developed Carabelli. Relative intercusp spacing is the primary factor that drives Carabelli formation and expression. More specifically, Carabelli expression is related to the spacing of specific cusps. The enamel knot that, mineralized, will become the protocone is the knot/cusp that ultimately controls the odds of Carabelli formation. My findings support this assumption. The partial correlation shows that only the intercusp distances between the paracone and protocone and between the protocone and metacone significantly correlate with Carabelli expression, with tooth size, population, and sex held constant. This finding fits the model's predictions geometrically.

My study also shows the importance of sex as a variable, which, along with relative intercusp average, is significantly related to Carabelli expression. This finding is not incompatible with the predictions of the model. If they differ between the sexes, certain developmental parameters could lead to changes in development and therefore differences in morphology and Carabelli expression between the sexes. Developmental research is needed to determine what parameters are involved in this relationship.

This type of study provides a unique look at cusp development not only in humans, but also in other mammals. Carabelli's cusp is only seen in its fully developed form in a select number of molars. The model therefore provides a diagram for the circumstances, both developmental and morphological, under which a new cusp may form and eventually become standard in a population or species, perhaps being a key distinguishing trait of such a group. Since the model was originally developed by experiments on mice and voles, this holds true for cusp development in various mammalian species. Carabelli's cusp provides a good example of general mammalian cusp development.

Cusp three-dimensionality and areas for future research

Not considered by this study due to technological limits, but a possible topic of future investigation, is the accountability of the enamel knot in three-dimensional space. This study models the three-dimensionality of a tooth on a two-dimensional plane. Teeth are dynamic, three-dimensional objects, and a new enamel knot cannot form until it has grown far enough from an existing knot in not just the x- and y-planes, but in the z-plane as well. The slope, or sharpness, of the epithelial tissue as it proliferates from an enamel knot therefore determines at what point a new knot can begin to form. This study was unable to account for this factor. The measurements of area and intercusp spacing are two-dimensional measurements, and therefore they are simply estimates of tooth size, Carabelli size, and intercusp distances. They cannot account for tooth and cusp height and for the three-dimensional spacing of cusps relative to one another. Three-dimensional modeling of teeth with Carabelli's cusp could certainly be useful for understanding the spatial factors that account for the formation of the cusp. It would also be interesting to investigate the effect of cusp sharpness on homoplasy, since shorter cusps should have higher degrees of homoplasy and trait flickering (Jernvall and Jung, 2000). Scanning or profiling technology with the ability to accurately measure three-dimensionality would provide an interesting follow-up to this study.

TABLES AND FIGURES

Table 1. Frequency of ASUDAS counts ordered by population and sex.

Population			Sex			Total
			Female	Male	Unknown	
Gullah	ASUDAS	0	15	3	0	18
		1	28	23	2	53
		2	21	8	0	29
		3	22	24	5	51
		4	10	9	3	22
		5	9	8	0	17
		6	0	2	0	2
		7	9	6	2	17
	Total		114	83	12	209
New York	ASUDAS	0	10	0	4	14
		1	10	3	8	21
		2	4	1	4	9
		3	9	5	7	21
		4	5	3	2	10
		5	0	2	1	3
		6	6	0	1	7
		7	0	3	2	5
	Total		44	17	29	90
Seminole	ASUDAS	0	16	5	-	21
		1	30	24	-	54
		2	10	18	-	28
		3	17	18	-	35
		4	13	4	-	17
		5	1	9	-	10
		6	10	8	-	18
		7	0	0	-	0
	Total		97	86	-	183
Total	ASUDAS	0	41	8	4	53
		1	68	50	10	128
		2	35	27	4	66
		3	48	47	12	107
		4	28	16	5	49
		5	10	19	1	30
		6	16	10	1	27
		7	9	9	4	22
	Total		255	186	41	482

Table 2. Actual counts and expected counts of ASUDAS frequency ordered by population.

			Population		Total
			Gullah	Seminole	
ASUDAS	0	Count	18	21	39
		Expected Count	20.8	18.2	39.0
	1	Count	53	54	107
		Expected Count	57.0	50.0	107.0
	2	Count	29	28	57
		Expected Count	30.4	26.6	57.0
	3	Count	51	35	86
		Expected Count	45.9	40.1	86.0
	4	Count	22	17	39
		Expected Count	20.8	18.2	39.0
	5	Count	17	10	27
		Expected Count	14.4	12.6	27.0
	6	Count	2	18	20
		Expected Count	10.7	9.3	20.0
	7	Count	17	0	17
		Expected Count	9.1	7.9	17.0
Total		Count	209	183	392
		Expected Count	209.0	183.0	392.0

Table 3. Results of proportional odds logistic regression with ASUDAS as response variable and population, sex, and relative intercusp average as explanatory variables.

Population coefficient	Sex coefficient	RIA* coefficient	Intercepts	LL (H1)	LL(H0)	G
-0.19571	0.428109	-4.71654	-4.86722	-716.115	-720.786	9.3426†
			-3.17135			
			-2.55397			
			-1.60117			
			-1.01731			
			-0.36377			
			-0.54796			

* = relative intercusp average
† = significant at level $P < 0.005$

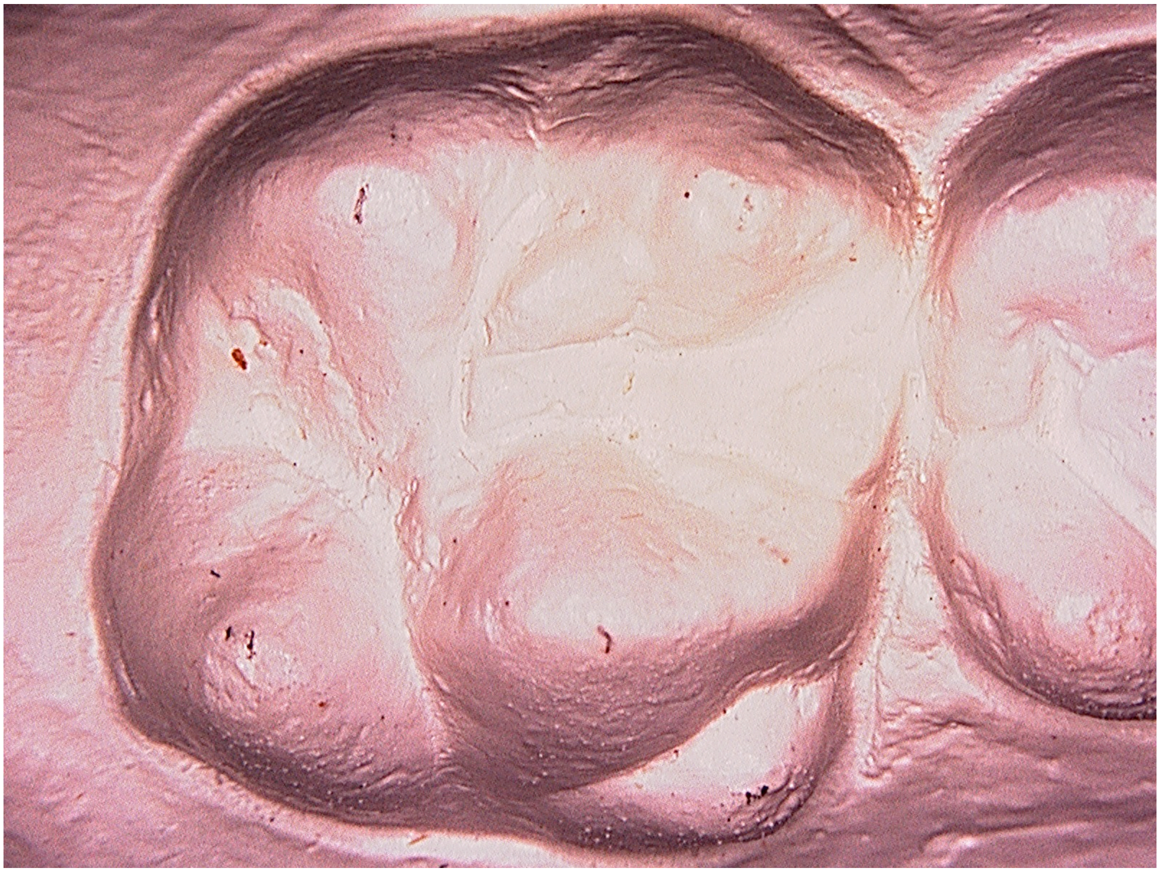
Table 4. Partial correlations between ASUDAS and individual intercusp distances with tooth area, population, and sex held constant as control variables.

		ASUDAS	paracone-protocone	paracone-metacone	metacone-protocone	paracone-hypocone	protocone-hypocone	metacone-hypocone
ASUDAS	Correlation	1.000	-.118	-.064	-.115	-.023	-.064	.023
	Significance†	.	.022	.215	.003	.652	.214	.662
	df	0	375	375	375	375	375	375
paracone-protocone	Correlation	-.118	1.000	.311	.576	.659	.047	.387
	Significance†	.022	.	.000	.000	.000	.364	.000
	df	375	0	375	375	375	375	375
paracone-metacone	Correlation	-.064	.311	1.000	.224	.500	.079	-.130
	Significance†	.215	.000	.	.000	.000	.125	.011
	df	375	375	0	375	375	375	375
metacone-protocone	Correlation	-.155	.576	.224	1.000	.310	.287	.558
	Significance†	.003	.000	.000	.	.000	.000	.000
	df	375	375	375	0	375	375	375
paracone-hypocone	Correlation	-.023	.659	.500	.310	1.000	.425	.480
	Significance†	.652	.000	.000	.000	.	.000	.000
	df	375	375	375	375	0	375	375
protocone-hypocone	Correlation	-.064	.047	.079	.287	.425	1.000	.066
	Significance†	.214	.364	.125	.000	.000	.	.202
	df	375	375	375	375	375	0	375
metacone-hypocone	Correlation	.023	.387	-.130	.558	.480	.066	1.000
	Significance†	.662	.000	.011	.000	.000	.202	.
	df	375	375	375	375	375	375	0

† = 2-tailed

Figure 1. Dr. Georg von Carabelli, an Austrian dentist after whom Carabelli's cusp is named.

Figure 2A. Picture of permanent upper right first molar with fully developed (ASUDAS = 7) Carabelli's cusp.



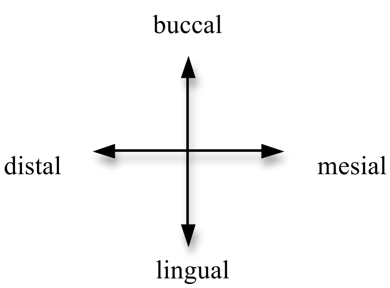
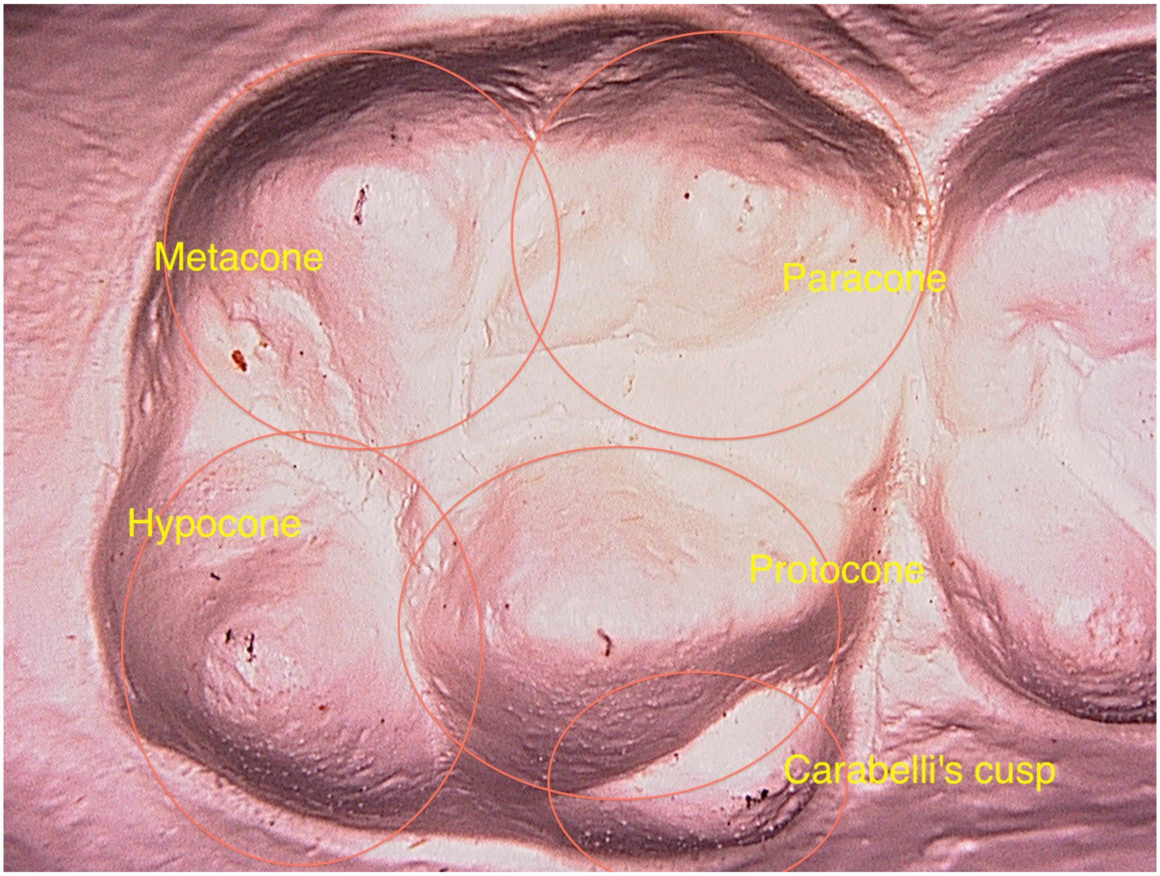


Figure 2B. Picture of permanent upper right first molar with fully developed (ASUDAS = 7) Carabelli's cusp with labeled cusps



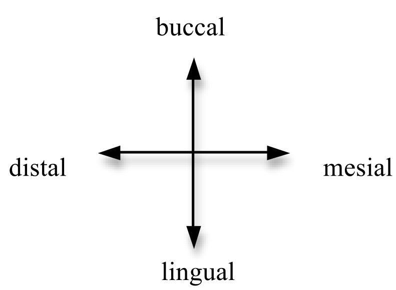


Figure 3. A plaque with the eight-point scale developed for the Arizona State University dental anthropology system to classify Carabelli development. Photograph by John P. Hunter.

Figure 4. Example of patterning cascade model of tooth morphogenesis and Carabelli formation. On two same-sized teeth, Carabelli's cusp is more likely to escape inhibitory signalers and form before cessation of growth on a tooth with closely spaced cusps. The Carabelli enamel knot is pictured without an inhibitory zone.

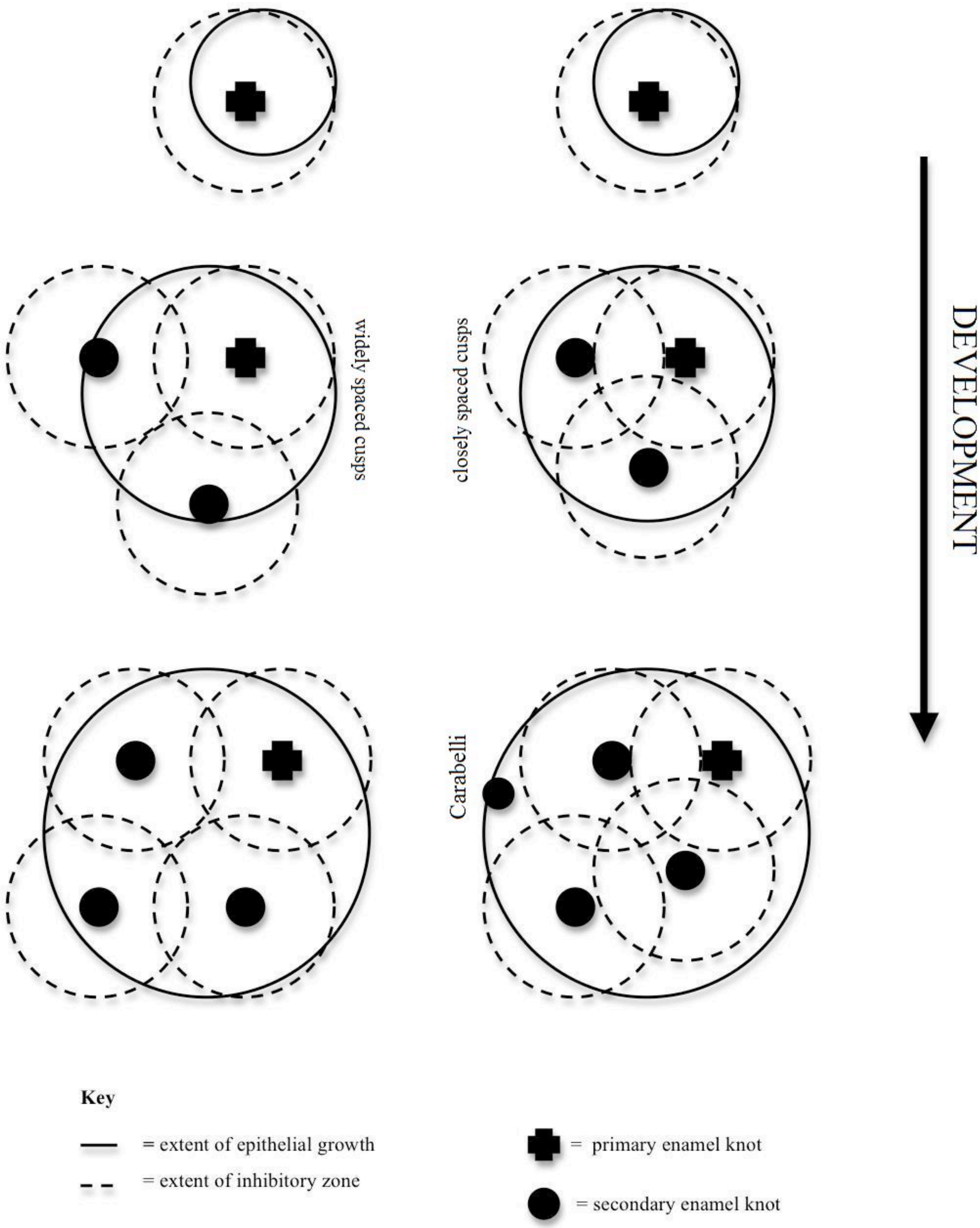


Figure 5. Expected distributions by tooth area and average intercusp distance for teeth by Carabelli development. From Hunter et al. 2010, Figure 1.

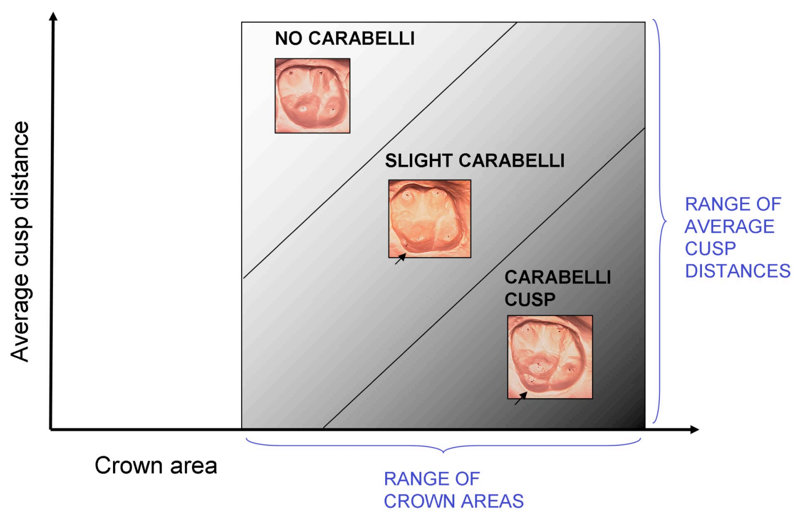
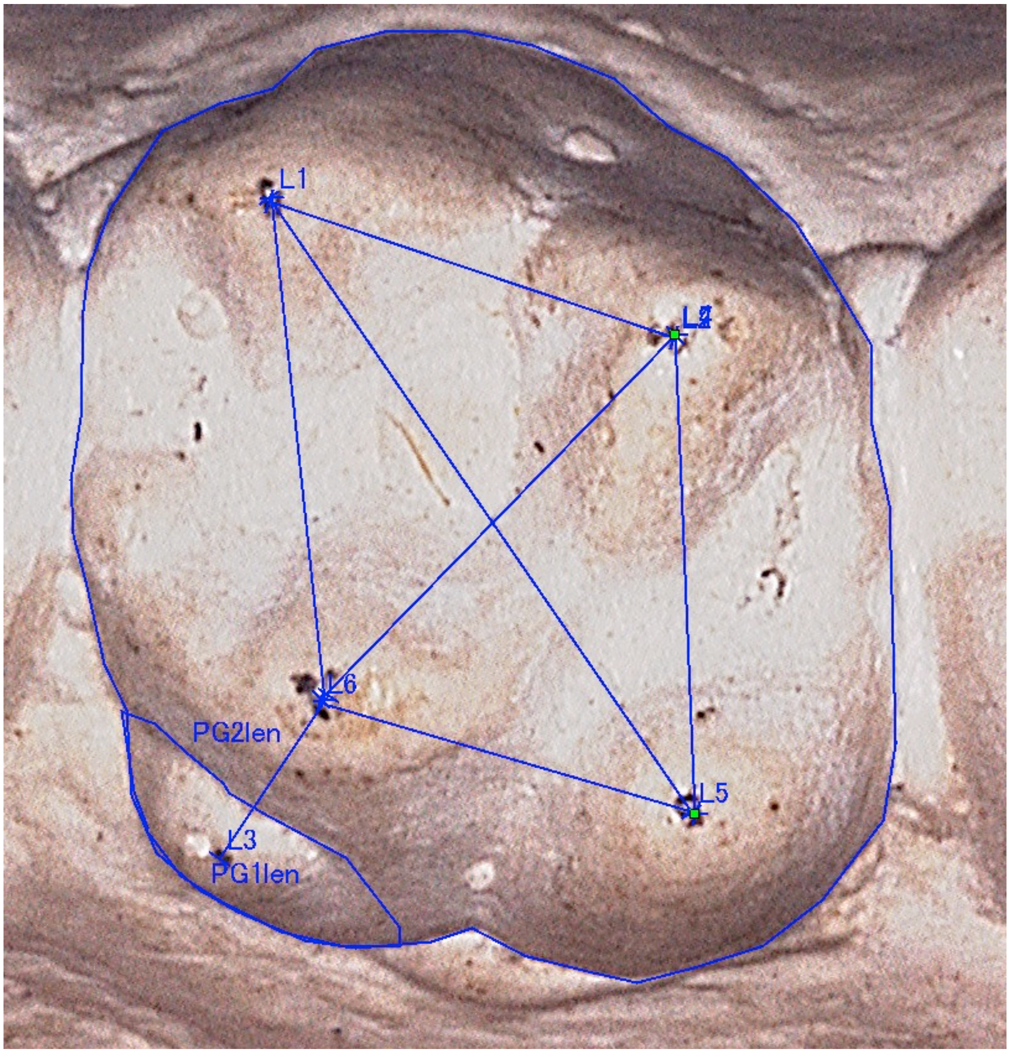


Figure 6. Two-dimensional measurements on a molar with a Carabelli's cusp using the Hirox digital microscope. Measurements can be seen for intercusp distance between the four main cusps, intercusp distance from the protocone to Carabelli's cusp, total area, and Carabelli area.



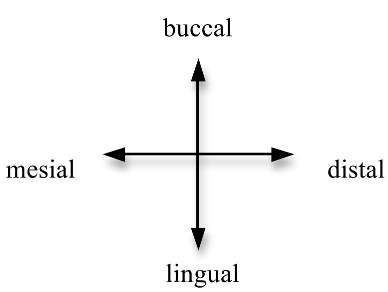


Figure 7. Box-and-whisker plot of the distribution of relative intercusp averages for teeth ordered by Carabelli development assessed as absent (ASUDAS = 0), slight (ASUDAS = 1 – 6), and present (ASUDAS = 7).

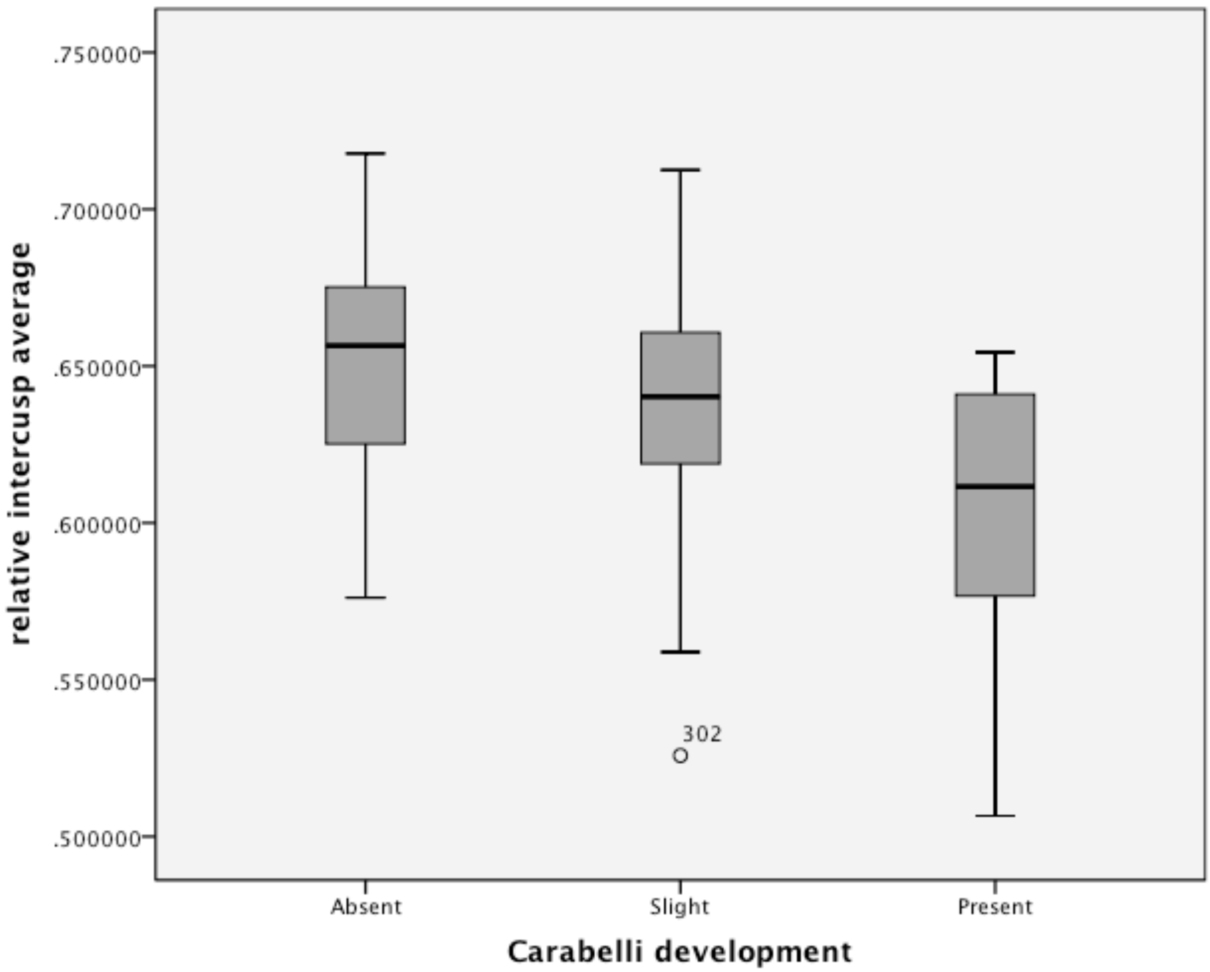


Figure 8. Plot of the relationship between square root tooth area and intercusp average. Teeth are labeled by Carabelli development as absent (ASUDAS = 0), slight (ASUDAS = 1 – 6), and present (ASUDAS = 7), with absent and present points emphasized for visual clarity.

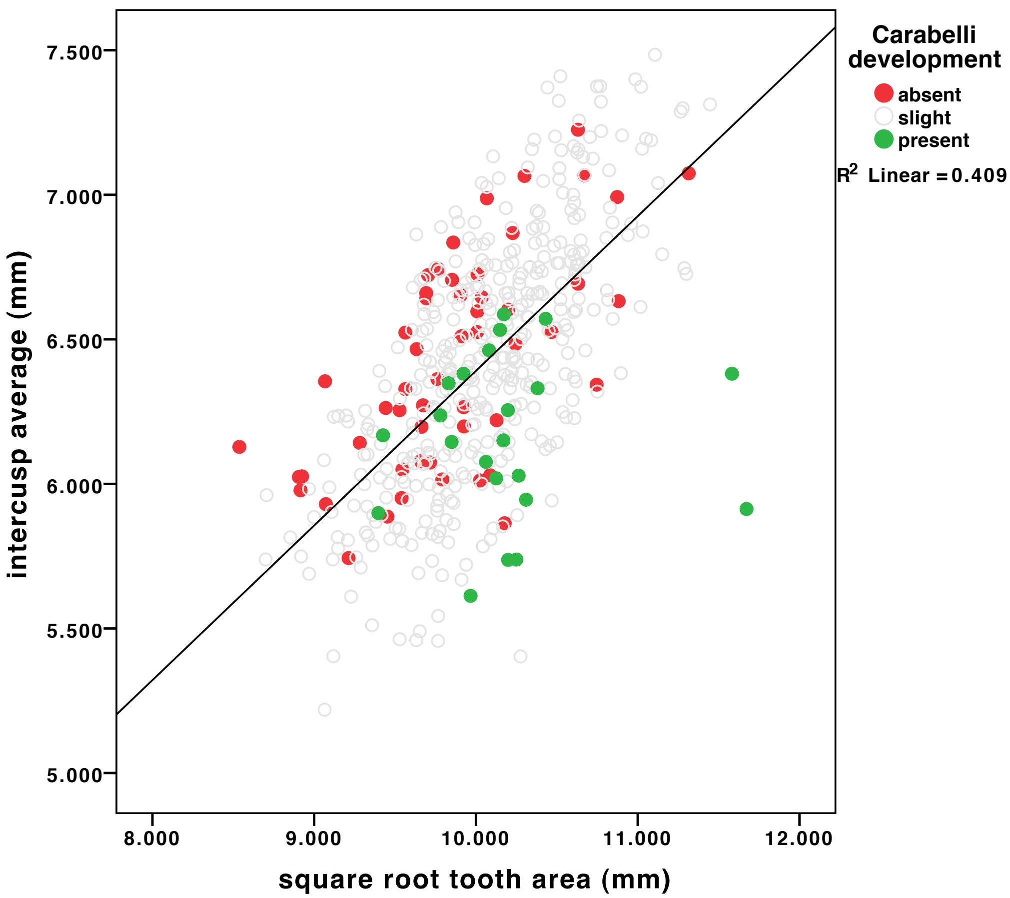


Figure 9. Scatter plot of relative intercusp average vs. ASUDAS with best-fit line showing the negative correlation between the variables.

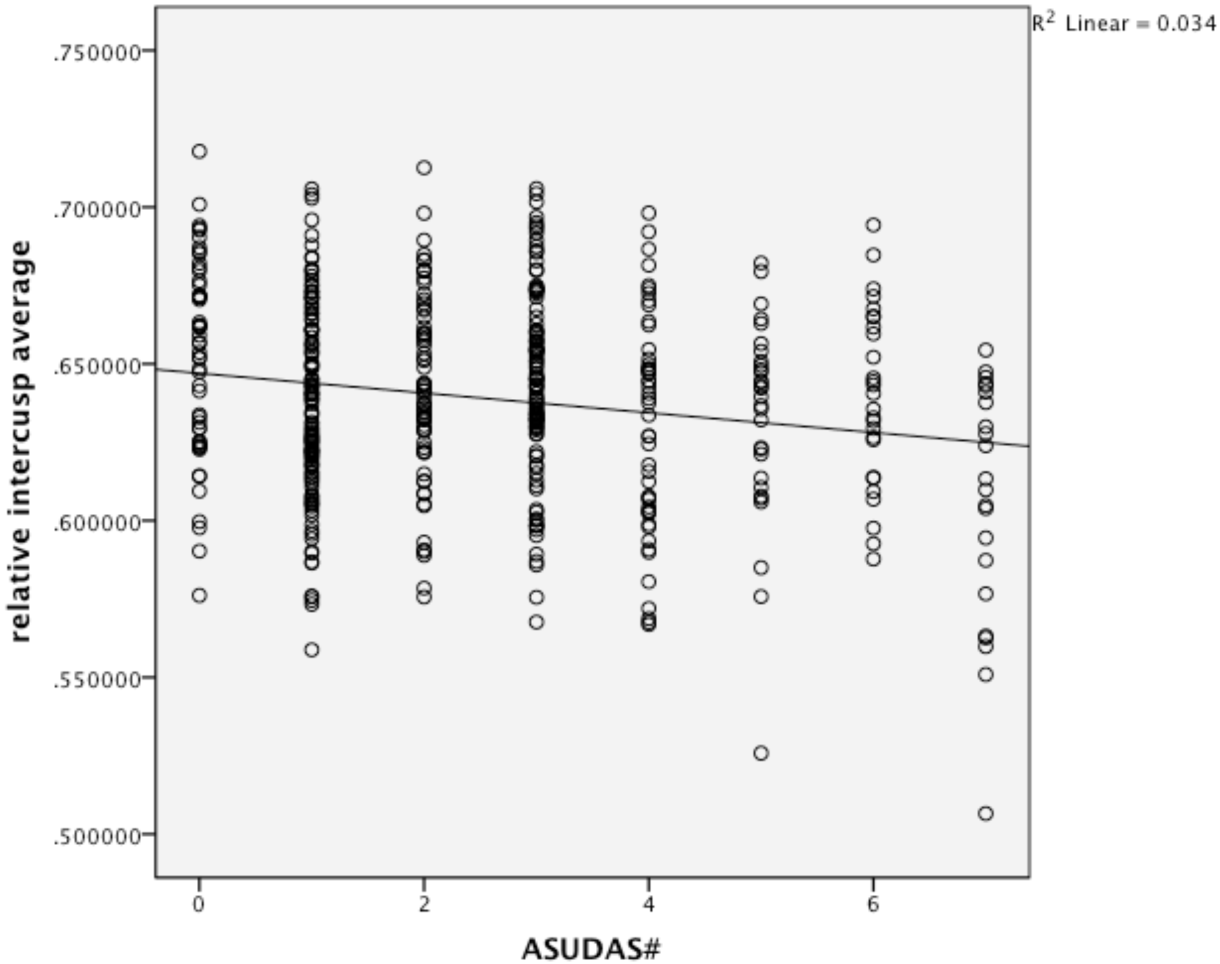


Figure 10. Scatter plot of square root Carabelli area vs. relative intercusp average with best-fit line showing the negative correlation between the variables.

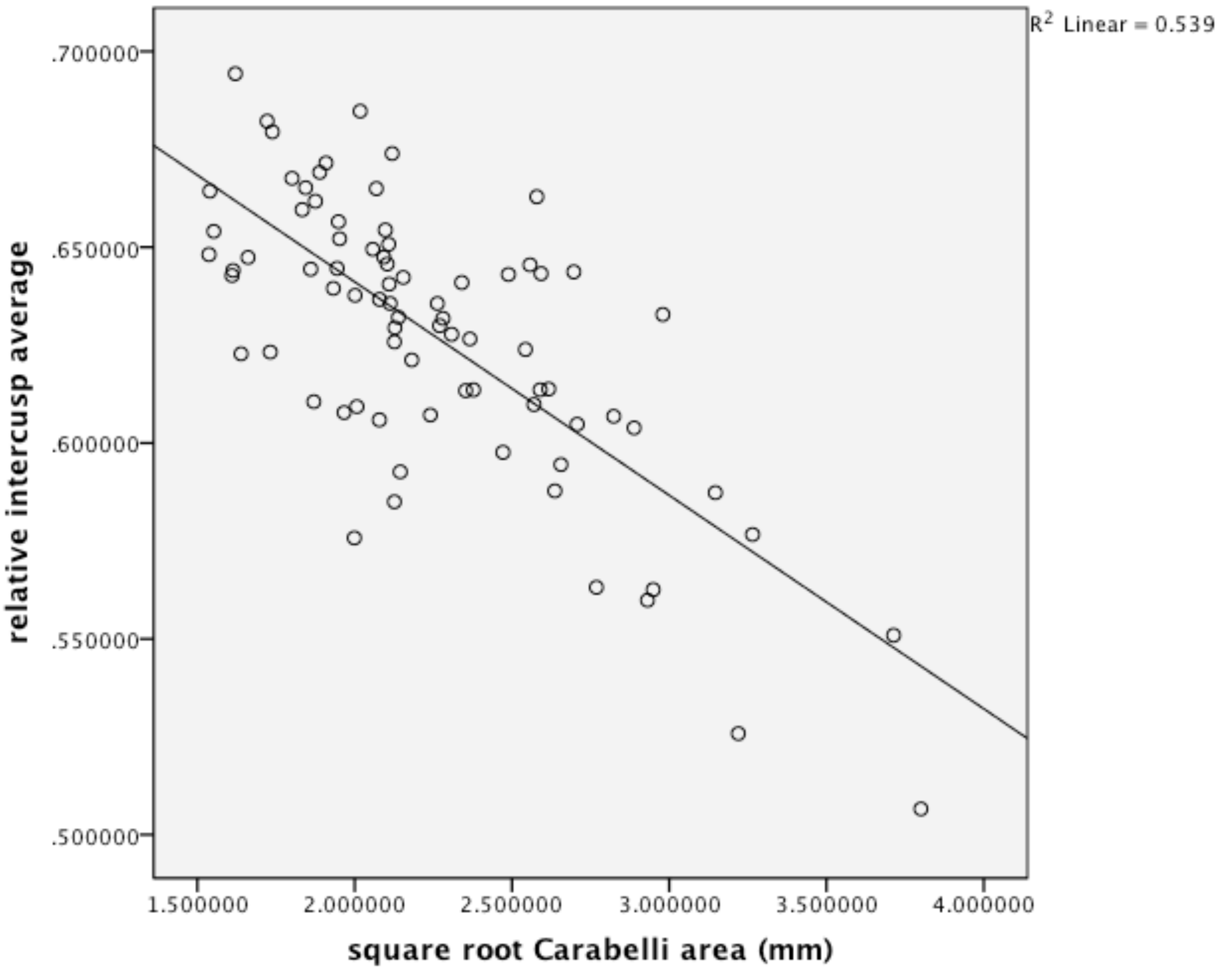
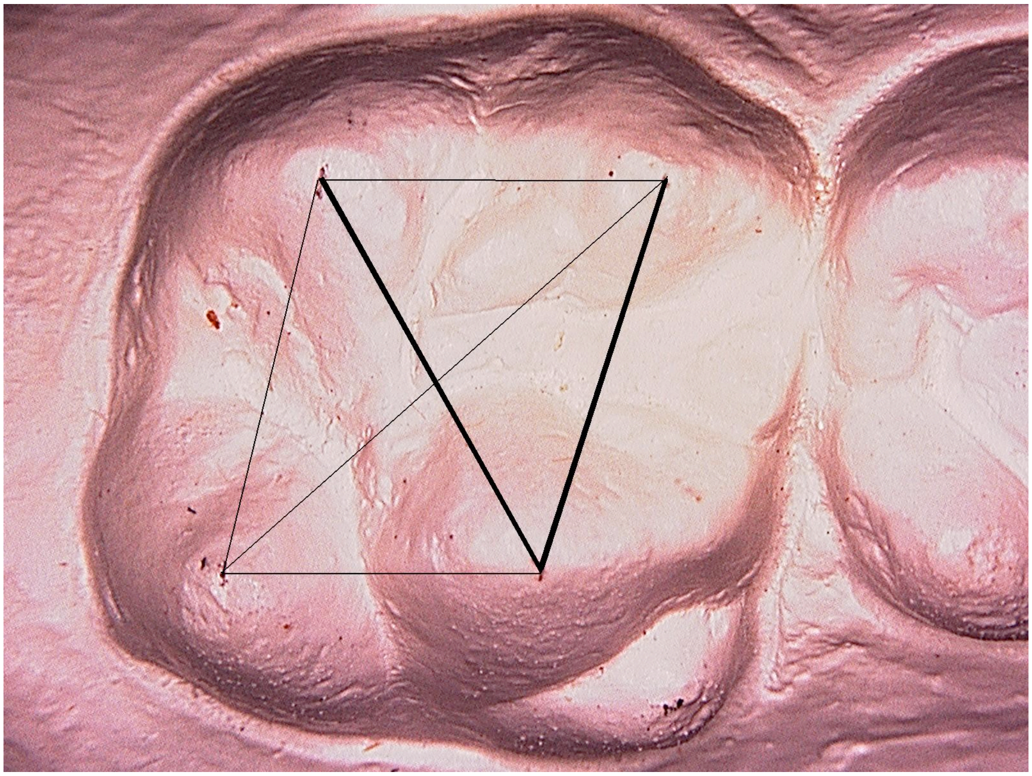


Figure 11. Intercusp distances shown in bold display significant negative correlations to Carabelli development, measured as ASUDAS, when total tooth area, sex, and population are held constant.



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